Research Article

An Insight in to the Efficacy of Dehydrozingerone against Aluminium Induced Cognitive Dysfunction in Rats

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ABSTRACT

Recent literatures favour the use of curcumin and its structural analogues in neurodegenerative disorders. The poor bioavailability of curcumin is a limitation to its ability to halt neurodegeneration. Thus the objective of our study was to assess the role of dehydrozingerone (DHZ), half analogue of curcumin in aluminium chloride induced cognitive dysfunction model in rats. The effects of two prophylactic doses of DHZ viz., 100mg/kg and 200mg/kg were assessed in aluminium treated rats by Morris water maze test. Aluminium treated rats showed decreased performance in the Morris water maze test. Rats treated with DHZ did not show improvement in cognition function in acquisition trials. The present study shows that prophylactic administration of DHZ (100mg/kg and 200mg/kg) did not improve the learning abilities against aluminium induced cognitive dysfunction in rats.

Keywords: Dehydrozingerone; Aluminium; Cognition; Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterised by neurological and cognitive dysfunctions[1]. The higher order cognitive skills like judgement, concentration, learning, memory, language skills and co-ordination are affected in AD patients[2]. AD is characterised by cognitive dysfunction due to the degeneration of neurons in different brain regions especially in hippocampus and frontal cortex regions[3,4].

Pathologically, AD is characterized by the accumulation of amyloid plaques and neurofibrillary tangles in the neuronal networks of memory formation and processing[5]. This progressive accumulation of insoluble senile plaques in the brain and intracellular accumulation of neurofibrillary tangles leads to degeneration of nerve cells in cerebral cortex and hippocampal formation.

This results in neuronal cell loss and changes in synapse which contribute to memory loss. Attack by free radicals is another contributing factor in AD[6].

Metals like iron, copper, zinc, and aluminium have catalytic activity that produces free radicals, which may lead to the formation of amyloid plaques[7]. β-amyloid aggregation is increased in presence of free radicals[8]. Further, reports suggest the ameliorative effect of free radical scavengers in β-amyloid toxicity[9].

Aluminium is a potent neurotoxic metal. Exposure of aluminium in animals is associated with behavioural, neuropathological and neurochemical changes[10].

Aluminium easily passes through CNS in normal physiological conditions and mount up in various areas of brain and thus responsible for cause of many neurodegenerative diseases. Aluminium has been suggested to generate reactive oxygen species damaging lipids, membrane proteins, and antioxidant enzyme system[11].

Curcumin, the main principle of turmeric, several properties of which have been proposed, includes antioxidant and anti-inflammatory activities[12]. Recently its ability to inhibit fibril formation *in vivo* and *in vitro* by direct binding to amyloid aggregates has been proposed[13-14]. The Aβ aggregation inhibition and destabilisation of Aβ peptide properties of curcumin contributes to its anti-dementia effect[15]. Further, literatures support the ability of curcumin to clear the amyloid plaques from the brain by phagocytosis[16]. But curcumin is insoluble in aqueous solution; it has poor bioavailability, and rapidly degrades at neutral or basic pH. All these problems limit the therapeutic use of curcumin. Dehydrozingerone, a natural compound extracted from the plant *Zingiber officinale* corresponding to half curcumin is one of its degradation by-product, but it is more water soluble and stable than curcumin[17]. In periphery, DHZ has been demonstrated to alleviate dexamethasone induced delay in wound healing[18]. Dehydrozingerone (DHZ) has been demonstrated to possess antioxidant and anti-inflammatory property as well[19]. Thus, the present study was designed to evaluate the role of dehydrozingerone in cognitive dysfunction model in rats.

MATERIALS AND METHODS

Synthesis of Dehydrozingerone

Vanillin, 5g, is the starting molecule for synthesis of dehydrozingerone. To this, added 40ml of acetone and 50ml of 0.5N sodium hydroxide solution with continuous stirring. The stirring was continued for 1 hour on magnetic stirrer. Then it was kept at room temperature to

allow the evaporation of excess acetone for 24hrs or more until there was very faint odour of acetone left. Now this solution was then acidified with 2N hydrochloric acid in ice cold condition and stirring, until yellow precipitate was formed. This precipitate was then dried and recrystallized with ethanol. Each batch of DHZ was analysed by Gas chromatography–mass spectrometry (GC-MS).

Experimental Animals

The protocol for the study was approved by the Institutional Ethics Committee (IAEC), Kasturba Medical College, Manipal University, Manipal, India. Male Wistar rats of 3 months old weighing 200-220g was procured from Central Animal Research Facility (CARF) under Manipal University, Manipal. These animals were maintained at room temperature (25°C) and relative humidity of 50-55%. They were given free access to feed and water and kept in polypropylene cages with sterile paddy husk bedding.

Experimental Design

$AlCl_3$ was prepared freshly each day in distilled water and given by intraperitoneal route at a dose of 10 mg/kg for a period of 42 days. This dose was selected based on our previous study which demonstrated the induction of cognitive dysfunction using $AlCl_3$[20]. Dehydrozingerone suspension was prepared in 0.5% CMC each day and given by oral route. Two doses of dehydrozingerone was used in the study viz., 100mg/kg and 200mg/kg. The dosing volume for each of the treatment group was 1ml/kg. Dehydrozingerone was administered forty five minutes before giving $AlCl_3$ for 42 days. Animals were randomised based on their body weight and divided in to four groups (n=6): Group 1: Control - 0.5% CMC (5ml/kg, p.o.), Group 2: $AlCl_3$ (10mg/kg, *i.p.*), Group 3: Dehydrozingerone (100mg/kg, *p.o.*) + $AlCl_3$ (10mg/kg, *i.p.*), Group 4: Dehydrozingerone (200mg/kg, *p.o.*) + $AlCl_3$ (10mg/kg, *i.p.*).

Behavioral Assessment

After forty two days of treatment, animals were assessed for their learning ability using Morris Water Maze test[21]. Morris Water Maze test is a behavioural procedure widely used in behaviour neuroscience to study spatial learning and memory. Water maze contains circular pool of 150 cm in diameter, comprising of water maintained at 25°C-27°C. There is a hidden platform for the animal to escape, which was made opaque with milk in order to hide the submerged platform. The temperature of water is much below the body temperature of the animal which is enough stressful to create a desire for animal to escape, without inhibiting the learning process. Visual clue was also placed in plain sight of the animal. The pool was divided in to four quadrants. The movement of the animal inside the pool was captured using a video camera and data were analysed using Any Maze (Ugo Basile, Italy) software. An Acquisition trial was conducted for four days. Each day four trials were given from four different quadrants. Duration of each trial was 60 sec with an inter-trial interval of 15s. When released into the pool, animal swims around in search of an exit, while various parameters are recorded. The parameters include escape latency (ELT) which is the time taken to reach the platform, average speed and total distance travelled. Initially there is mild stress reaction to escape from water which motivates it to escape quickly to the platform and on subsequent trails the performance increases which occurs as a result of learning.

Statistical Analysis

Data are expressed as mean ± SEM. To compare between the groups, acquisition trial of day1 and day4, two-way ANOVA followed by Bonferroni's post hoc test was employed.

RESULTS AND DISCUSSION

Behavioural study-Morris water maze

Escape latency

After forty two days of aluminium treatment, ELT of the animals showed significant decrease as compared to the control group. Prophylactic treatment with DHZ (100mg/kg, 200mg/kg) did not alter the effects of $AlCl_3$. Eventhough DHZ animals showed improved ELT than $AlCl_3$ rats, data was insignificant.

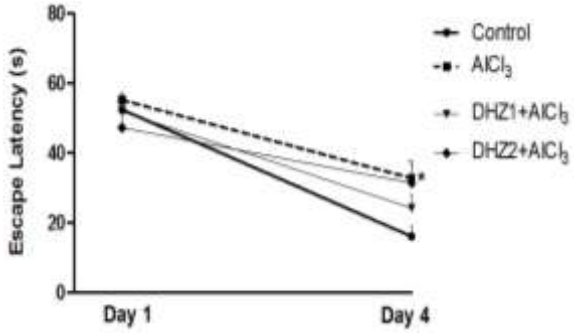

Figure 1: Escape latency of treatment groups ($AlCl_3$, DHZ1- Dehydrozingerone 100mg/kg, DHZ2- Dehydrozingerone 200mg/kg) during acquisition trial. Values are expressed as mean ± SEM (n=6). *P<0.05 as compared to control group.

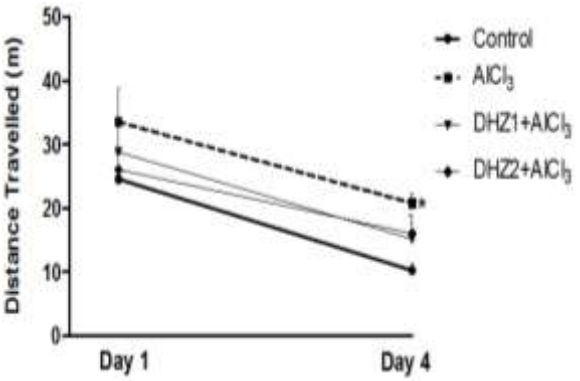

Figure 2: Distance travelled to target of treatment groups ($AlCl_3$, DHZ1- Dehydrozingerone 100mg/kg, DHZ2- Dehydrozingerone 200mg/kg) during acquisition trial.

Values are expressed as mean ± SEM (n=6). *P<0.05 as compared to control group.

Distance travelled to target

In acquisition trials, distance travelled to target is measured as an index of learning. In $AlCl_3$ treated rats, distance travelled was significantly increased as compared to control. Treatment with 100mg/kg and 200mg/kg of DHZ has shown a trend towards reducing the debilitating effects of $AlCl_3$ as evidenced by improvement in distance travelled to target as compared to $AlCl_3$ group.

Average speed

Average speed of the animals in water maze serves as an index of locomotion. There was no significant change observed in average speed between the treatment groups.

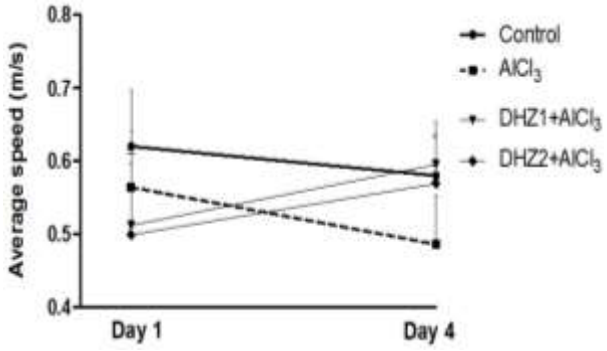

Figure 3: Average speed of treatment groups ($AlCl_3$, DHZ1-Dehydrozingerone 100mg/kg, DHZ2- Dehydrozingerone 200mg/kg) during acquisition trial. Values are expressed as mean ± SEM (n=6).

Alzheimer's disease is the most common cause of mental deterioration in elderly people. Evidences are suggestive of acetylcholine releasing neurons which lie in the basal forebrain, degenerating selectively in AD. These cholinergic neurons present in cerebral forebrain appear to have an important role in cognitive functions, especially in memory. Basal forebrain cholinergic cell loss is a consistent feature of AD. This has indicated to contribute to β-amyloid plaque formation, hyperphosphorylation of tau protein[22]. Neurons and glial cells together have a role in brain function. Glial cells under normal physiological conditions provide neurotrophic factors required for neurogenesis. Further, glial cells are activated under stressful conditions to produce excessive cytokines leading to neuroinflammation[23].

Aluminium is a neurotoxic metal that gains easy access to the central nervous system and accumulates in different parts of brains. It also causes oxidative stress related damage to lipids and endogenous antioxidant enzyme activity[24]. The long term exposure of AlCl3 has been found to have neuro degenerative effect resulting in learning deficits[20]. Aluminium levels have been found at elevated levels in neurons containing neurofibrillary tangles and the changes followed with neuro pathological and behavioural changes were similar to those of observed in AD[25]. In the present study, $AlCl_3$ was found to delay the acquisition parameters evidenced by significant increase in ELT and distance travelled to target. Acquisition reflects the learning ability of animals[26]. As the acquisition trials progress from day 1 to day 4 animals develops a mapping strategy to reach the hidden platform which is linked with the long-term potentiation and excitatory neurotransmission in the synapse. The increase in the time taken and distance travelled to find the platform in aluminium rats indicate the neurotoxic effect of aluminium deleteriously affecting the synaptic plasticity. Our reports were consistent with the previous reports suggesting the decreased performance of aluminium treated rats in morris water maze[20,27]. The neurotoxic effects of aluminium could be linked to its ability to generate insoluble amyloid plaques and oxidative stress[24].

The two doses of dehydrozingerone used in this study were 100mg/kg and 200mg/kg. The dose of 100mg/kg was found to have antioxidant and anti-inflammatory role in a study conducted in wound healing models in rats[18]. Further, a higher dose (200mg/kg) was also employed in this study. We hypothesized that the antioxidant, anti-inflammatory and better bioavailability of dehydrozingerone may result in a neuroprotective action against aluminium mediated cognitive dysfunction. As indicated by increased ELT and distance travelled to target in acquisition trial, both the doses were not able to overcome the devastating effect of $AlCl_3$ on cognition. One of the limitations of our study was that retention trial was not conducted to evaluate the effect of treatment on memory.

We agree that reasons for the lack of anti-dementia activity of DHZ are difficult to explain with this present data. We speculate that inability of DHZ to show cognitive improvement effect might be attributed to dose used, route of administration, prophylactic mode of administration, pharmacokinetic parameters, and specificity of drug to accumulate in different brain regions. Further, dose-dependent effects of DHZ correlating learning and memory specifically with brain antioxidant and anti-inflammatory parameters are warranted to clarify the role of dehydrozingerone in AD.

CONCLUSION

The current study indicates that prophylactic administration of DHZ (100mg/kg and 200mg/kg) did not improve the learning abilities in aluminium induced cognitive dysfunction in rats.

REFERENCES

1. Kawahara M, Kato M, Kuroda Y. Effects of aluminum on the neurotoxicity of primary cultured neurons and on the aggregation of beta-amyloid protein. Brain Research Bulletin, 55(2), 2001, 211-217.
2. Gerstadt CL, Hong YJ, Diamond A. The relationship between cognition and action: performance of children 312–7 years old on a stroop-like day-night test. Cognition, 53(2), 1994, 129-53.
3. Buckner RL. Frontal cortex contributes to human memory formation. Nature Neuroscience, 4, 1999, 311-4.
4. Pennanen C, Kivipelto M, Tuomainen S, Hartikainen P, Hanninen T, Laakso MP, Hallikainen M, Vanhanen M, Nissinen A, Helkala EL, Vainio P. Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. Neurobiology of aging. 25(3), 2004, 303-10.
5. Paulson JB, Ramsden M, Forster C, Sherman MA, McGowan E, Ashe KH. Amyloid plaque and neurofibrillary tangle pathology in a regulatable mouse model of Alzheimer's disease. The American journal of pathology. 173(3), 2008, 762-72.
6. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature. 443(7113), 2006, 787-95.
7. Lin R. Exposure to metal ions regulates mRNA levels of APP and BACE1 in PC12 cells: blockage by curcumin. Neuroscience Letters 3, 2008, 344-7.
8. Butterfield DA. β-Amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. Chemical research in toxicology. 10(5), 1997, 495-506.
9. Dore S, Bastianetto S, Kar S, Quirion R. Protective and Rescuing Abilities of IGF-I and Some Putative Free Radical Scavengers against β-Amyloid-Inducing Toxicity in Neurons. Annals of the New York Academy of Sciences. 890(1), 1999, 356-64.
10. Luo Y, Nie J, Gong QH, Lu YF, Wu Q, Shi JS. Protective effects of icariin against learning and memory deficits induced by aluminium in rats. Clinical and experimental pharmacology and physiology. 34(8), 2007, 792-5.
11. Kumar V, Gill KD. Aluminium neurotoxicity: neurobehavioural and oxidative aspects. Archives of toxicology. 83(11), 2009, 965-78.
12. Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radical Biology and Medicine. 28(8), 2000, 1303-12.
13. Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, Chen PP, Kayed R, Glabe CG, Frautschy SA, Cole GM. Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid in vivo. Journal of Biological Chemistry. 280(7), 2005, 5892-901.
14. Ono K, Hasegawa K, Naiki H, Yamada M. Curcumin has potent anti-amyloidogenic effects for Alzheimer's β-amyloid fibrils *in vitro*. Journal of neuroscience research. 75(6), 2004, 742-50.
15. Airoldi C, Zona C, Sironi E, Colombo L, Messa M, Aurilia D, Gregori M, Masserini M, Salmona M, Nicotra F, La Ferla B. Curcumin derivatives as new ligands of Aβ peptides. Journal of biotechnology. 156(4), 2011, 317-24.
16. Cole GM, Teter B, Frautschy SA. Neuroprotective effects of curcumin. In The molecular targets and therapeutic uses of curcumin in health and disease 2007, 197-212. Springer US.
17. Kim SJ, Kim HM, Lee ES, Kim N, Lee JO, Lee HJ, Park NY, Jo JY, Ham BY, Han SH, Park SH. Dehydrozingerone exerts beneficial metabolic effects in high-fat diet-induced obese mice via AMPK activation in skeletal muscle. Journal of cellular and molecular medicine. 19(3), 2015, 620-9.
18. Rao MC, Sudheendra AT, Nayak PG, Paul P, Kutty GN, Shenoy RR. Effect of dehydrozingerone, a half analog of curcumin on dexamethasone-delayed wound healing in albino rats. Molecular and cellular biochemistry. 355(1-2), 2011, 249-56.
19. Rajakumar DV, Rao MN. Antioxidant properties of dehydrozingerone and curcumin in rat brain homogenates. Molecular and cellular biochemistry. 140(1), 1994, 73-9.
20. Khan KA, Kumar N, Nayak PG, Nampoothiri M, Shenoy RR, Krishnadas N, Rao CM, Mudgal J. Impact of caffeic acid on aluminium chloride-induced dementia in rats. Journal of Pharmacy and Pharmacology. 65(12), 2013, 1745-52.
21. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. Journal of neuroscience methods. 11(1), 1984, 47-60.
22. Burke MA, Apter JR, Wainer BH, Mufson EJ, Kordower JH. Age-related vulnerability of developing cholinergic basal forebrain neurons following excitotoxic lesions of the hippocampus. Experimental neurology. 128(2), 1994, 159-71.
23. Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. Neurogenesis in the adult human hippocampus. Nature medicine. 4(11), 1998, 1313-7.
24. Nampoothiri M, John J, Kumar N, Mudgal J, Nampurath GK, Chamallamudi MR. Modulatory role of simvastatin against Aluminium chloride-induced behavioural and biochemical changes in rats. Behavioural neurology. 2015, 1-8.
25. Buraimoh AA, Ojo SA, Hambolu JO, Adebisi SS. Effects of Aluminium Chloride on Anxiety-Related Behaviour. American Journal of Neuroscience. 2(2), 2011, 65-9.
26. Ramalingayya GV, Nampoothiri M, Nayak PG, Kishore A, Shenoy RR, Rao CM, Nandakumar K. Naringin and rutin alleviates episodic memory deficits in two differentially challenged object recognition tasks. Pharmacognosy Magazine. 12 (Suppl 1), 2016, S63.
27. John J, Nampoothiri M, Kumar N, Mudgal J, Nampurath GK, Chamallamudi MR. Sesamol, a lipid lowering agent, ameliorates aluminium chloride induced behavioral and biochemical alterations in rats. Pharmacognosy magazine. 11(42), 2015, 327.

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